Bone Lead as a Biological Marker in Epidemiologic Studies of Chronic Toxicity: Conceptual Paradigms

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The skeleton contains the majority of the body's lead burden in both children and adults. The half-life of lead in bone is in the range of years to decades, depending on bone type, metabolic state, and subject age, among other things. Measurement of skeletal lead has benefited greatly from the recent development of X-ray fluorescence (XRF) instruments that can make rapid, safe, accurate, and relatively precise measurements of lead in bone. Two types of XRF technologies exist, LXRF and KXRF; this paper focuses on KXRF, which has been the most widely validated and used. KXRF is proving to be a powerful analytical methodology for evaluating bone lead levels as a measure of time-integrated (i.e., cumulative) lead dose in epidemiologic studies of the effects of chronic lead exposure. However, insufficient attention has been given to conceptualizing the paradigms by which bone lead levels reflect lead exposure and by which the skeleton serves as an endogenous source of lead. Consideration of these paradigms, which rely on bone lead kinetics, is necessary for the proper development of *a priori* hypotheses involving bone lead accumulation and release, the selection of bone sites for measurement by KXRF, and the design of epidemiologic studies involving bone lead dynamics. We discuss and present supporting evidence for a conceptual model that distinguishes two major paradigms of skeletal lead, including 1) bone lead as an indicator of cumulative lead exposure (bone lead as repository), and 2) bone lead as a source of body lead burden that is mobilizable into the circulation (bone lead as source). These two roles are not mutually exclusive. Instead, they are components of the processes controlling lead accumulation into and release from bone over time. Developing successful strategies for distinguishing these two processes in epidemiologic studies will require separate measurements of lead in cortical and trabecular bone and additional measurement of specific markers of bone mineral turnover and resorption. It may also involve developing accurate methods for evaluating lead in labile compartments of the circulation, such as plasma, as a potentially useful and responsive measure of bone lead release, of the partitioning of circulatory lead, and of the toxicological significance of lead released from bone to other target organs. *Key words*: blood, bone, lead, osteoporosis, plasma, X-ray fluorescence.
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Beginning in the 1970s, as the scientific community compiled evidence of adverse health outcomes associated with lead, a number of federal regulatory and legislative efforts were undertaken to reduce lead hazards in the United States, including actions to limit or eliminate the use of lead in paint, solder, and gasoline together with the voluntary efforts by the food processing industry (*1*). These efforts have been successful in part, as reflected by the 72–77% decline in mean blood lead levels of children surveyed in the 1976 and 1991 National Health and Nutrition Examination Surveys II and III, respectively (*2*).

Nevertheless, concern remains high regarding lead exposure and health due, in part, to the continued high exposure of specific sociodemographic groups and the recent accumulation of studies indicating significant relationships between low-level lead exposure and neurologic toxicity in both children and adults. For example, low to moderate blood lead levels (i.e., above 10 but less than 25 µg/dl) have consistently been found to be associated with decrements in cognitive functioning across a wide variety of epidemiologic studies of children (*1,3–5*). With regard to adults, low to moderate levels of lead in blood have been associated with increases in blood pressure [as reviewed by Sharp et al. (*6*), Hertz-Picciotto and Croft (*7*), and Schwartz (*8*)] as well as decreased creatinine clearance (*9,10*). These studies suggest that the prevalence of subclinical lead toxicity in both children and adults is far more prevalent than commonly recognized.

Is Blood Lead the Best Biological Marker for Predicting Toxicity?

In reviewing clinical studies on the effects of low to moderate lead exposures, it is noteworthy that the biological marker of lead dose that has been used most commonly was the level of lead in blood, largely because blood lead sampling is recognized as a relatively easy procedure. Lead in blood possesses a mean biological life of only around 30 days (*11*); thus, it has been generally considered to reflect primarily both ongoing steady-state exposures and relatively recent elevated exposures, rather than cumulative dose. Blood lead levels also reflect the mobilization of lead from the skeleton back into the circulation. This has been reported in the occupational setting (*12*), clinical studies (*13*), among women with community exposure (*14*), among pregnant women (*15*), and among adults undergoing joint replacement (*16*). Thus, blood lead serves as an indicator of both current exogenous exposures and past exposures as stored in the skeleton.

While many studies have detected significant relationships between blood lead level and toxicity outcomes, the correlations generally have been statistically weak and the effect parameters relatively small [for example, see Pocock et al. (*17*) on the relationship of blood lead to intelligence in children and Hertz-Picciotto and Croft (*7*) on the relationship of blood lead to blood pressure]. This may be because the relationships involved are 1) biologically weak or experienced by only a small segment of the populations studied; 2) biologically irrelevant and only found because of uncontrolled confounding by another more causal but unmeasured factor; 3) multifactorial, whereby the exposure–toxicity axis is mediated or

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modified by numerous inherent biologic factors that may be poorly recognized and/or quantitated; or 4) because blood lead level is not a sufficiently sensitive marker of exposure or dose to the target organs(s) for many outcomes.

In the latter case, the error associated with using blood lead as a proxy for a biologically more relevant biomarker of lead dose would, under most circumstances, result in weak correlations and small effect estimates in linear regression analyses (*18*).

In fact, a critical mass of indirect and direct evidence now suggests that a cumulative measure of lead dose (i.e., exposure that is integrated over many years of time), rather than blood lead level, may be the most important determinant of some forms of toxicity (*19,20*). Much of these data will be reviewed herein. As a consequence, there is a great need in public health sciences for the development of biologic markers that reflect a subject's cumulative dose (defined here as the lead dose integrated over years to decades).

K X-Ray Fluorescence

Until recently, epidemiologic research on whether cumulative lead dose is the most important determinant of low-level lead toxicity has been hampered by the lack of a suitable noninvasive and convenient method for estimating the cumulative lead dose of an individual. Shed primary teeth have proven to be a reliable measure of cumulative lead dose, but they are only suitable for children at certain ages, severely limiting their application (*21*). The calcium–EDTA mobilization test requires parenteral administration of a drug, collection of urine for hours to days, and monitoring for drug toxicity.

However, X-ray fluorescence (XRF) instruments, which make *in vivo* measurements of lead levels in bone, are quite promising in this regard (*19,22,23*). It has been well established from autopsy studies that the skeleton contains the majority (70–>95%) of the body's lead burden (*24,25*). The bulk of that lead is contained within long-lived compartments of cortical (elimination $t_{1/2}$>5–10 years) and trabecular (elimination $t_{1/2}$ >1 year) bone, with comparatively small amounts of lead in tissue compartments that rapidly exchange with extracellular fluid and plasma (*12,13,26–28*). Thus, measurement of bone lead levels may provide a surrogate of time-integrated lead exposure.

Two types of XRF instruments exist, LXRF and KXRF. This paper focuses on KXRF, which has been the most widely used and validated technology. Discussion and critiques of LXRF may be found elsewhere (*29–31*).

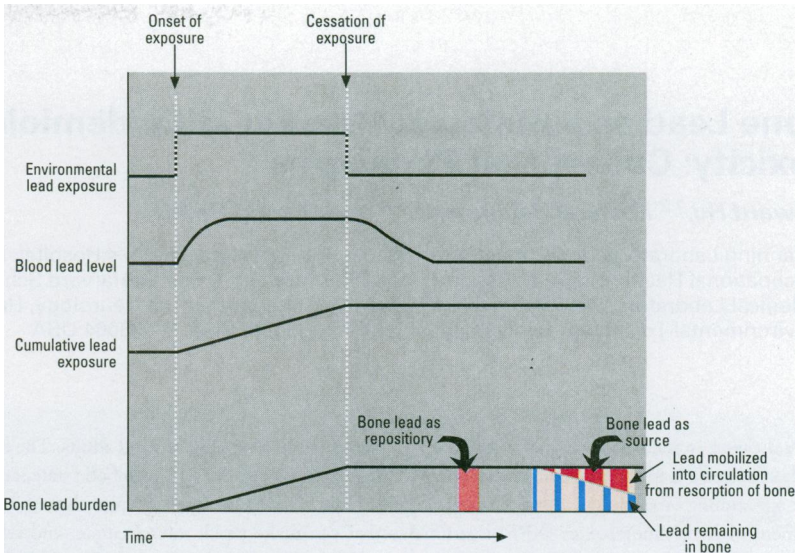

Figure 1. Conceptual paradigms of how bone lead may serve as a marker of chronic lead toxicity in adults: bone lead as repository versus bone lead as source (profile for blood lead level considers only exogenous sources of lead exposure).

Most KXRF instruments use a ^{109}Cd or ^{57}Co γ-ray source to provoke the emission of fluorescent photons from target tissues that are then detected and counted. With specialized equipment, designs, statistical methods, and software, methods have been developed to derive estimates of bone lead (in units of micrograms of lead per gram bone mineral) with ever-improving precision and accuracy (*32–34*). Radiation doses are minimal, with effective doses a small fraction of those associated with standard radiographs (*35*).

Thus, KXRF-measured bone lead has the potential to serve as a new indicator of dose that may be of greater relevance to many toxicity outcomes than blood lead level. This potential has been substantiated in four recent cross-sectional studies. In one of these studies, bone lead, but not blood lead, was significantly related to declines in hematocrit and hemoglobin among moderately lead-exposed construction workers (*36*). Among middle-aged to elderly men, bone lead, but not blood lead, was significantly related to increased odds of clinically relevant hypertension (*37*). Bone lead, but not blood lead, was significantly related to decreased birth weight among mothers in Mexico City (*38*). And finally, among teenagers in Pittsburgh, bone lead was associated with increased risk for antisocial and delinquent behavior (blood lead was not measured in this study) (*39*).

Other toxicity outcomes that may bear greater association with bone lead levels (as a time-integrated measure of exposure) include cognitive performance and growth in children and cognitive performance, kidney function, gout, blood pressure, reproductive toxicity, and adverse cardiovascular events in adults. A number of epidemiologic studies are now in progress using KXRF to measure bone lead to evaluate some of these associations, and more are being proposed, planned, and contemplated.

Conceptual Paradigms

Given the potential utility of XRF in epidemiologic studies, it is vital to develop a conceptual paradigm of the relationships between bone lead levels and toxicity outcomes. Such a paradigm would benefit from the use of bone lead measurements in epidemiologic studies by identifying toxicity outcomes that may be predicted by bone lead level (as a surrogate of cumulative exposure), as well as the selection of bone sites for KXRF measurement and the design of epidemiologic studies involving bone lead.

We propose that lead in the skeleton may serve two general roles as a marker of chronic lead toxicity in adults. One paradigm is that lead stored in bone presents no toxic risk to the individual, but rather, by being a repository for lead, serves as a proxy for the cumulative (i.e., over years to decades) lead dose to organs, such as the renal and central nervous systems, that are primary targets of lead toxicity (bone lead as repository) (Fig. 1). Conceptually, the bone lead as repository paradigm provides that bone lead levels would serve as a more relevant dosimeter of lead exposure and effect in cases of past (though not current) chronic lead exposures. In such cases, toxic effects may be expected to be greater than would be predicted by current blood lead levels.

A second paradigm is that the skeleton is an important endogenous source of lead to

other target organs via mobilization from bone into the circulation (bone lead as source) (Fig. 1). The release of lead from the skeleton could be a consequence of normal lead flux (exchange) from bone matrix, though it could be accelerated during periods of increased bone turnover and bone mineral loss. This may result in relatively prolonged elevated exposures and possibly also serve as a source of more acute exposure in cases where bone lead levels are elevated.

With either paradigm, the relationship between exposure and toxicity could involve several different aspects of dose–time response, such as direct linear, threshold, or threshold plus a period of latency. Finally, it should also be considered that the skeleton itself may be an important target organ to circulating and skeletal-bound lead.

While it is likely that both of the paradigms presented above are operant, distinguishing their relative importance provides significant implications for public health. If the bone lead as repository paradigm dominates, measurement of bone lead would provide an estimation of cumulative lead dose, but bone lead itself would be of little toxicological importance. In contrast, if the bone lead as source paradigm dominates, important increases in lead exposure from endogenous (i.e., skeletal) sources could occur so that chronic lead toxicity might be increased by factors that increase bone turnover, such as rapid growth [e.g., childhood (*27,40*), pregnancy and lactation (*15,41–44*), senile osteoporosis (*45,46*), altered endocrine states such as hyperthyroidism (*47*), and other pathological states (*48*)]. Alternatively, chronic lead toxicity might be decreased by factors that decreased bone turnover, such as exercise and dietary calcium (*49,50*). It would be critically important to identify and measure the influence of these factors, particularly since it might then be possible to develop strategies for the secondary prevention of toxicity from mobilized bone lead.

Evidence for bone lead as repository versus bone lead as source. Teeth are mineralized tissue with properties similar to bone. Thus, studies which demonstrate that lead levels in shed deciduous teeth are superior to blood lead levels in predicting neurobehavioral impairment in children (*51*) provide support for the bone lead as repository paradigm. Additional evidence linking measurable toxicity with bone lead level as the measure of exposure is provided in studies where lead-related pathologies have been associated with previous chronic exposures rather than with an ongoing insult. Examples include the focal interstitial nephritis and tubular atrophy of lead nephropathy (*52*) and the reduced density of synaptic formation, reduced dendritic arborization, and abnormal Purkinje cell development associated with lead exposure and neurotoxicity in animal models (*53–55*). However, these and other morphologic changes, including changes with a chronic appearance, have yet to be described in subjects that have sustained relatively moderate lead exposures (i.e., blood lead levels of 25–40 μg/dl).

The second paradigm is based on the belief that the skeleton is an endogenous source of lead to the circulation. As indicated previously, clinical and experimental studies have provided evidence of significant bone lead release during both normal homeostasis (*13,41,56*) and following the cessation of chronic occupational exposures (*12,57*), as well as during periods of accelerated bone turnover and mineral loss, such as osteoporosis, pregnancy, and lactation (*41–45*) and thyroid and parathyroid hormone imbalances (*46,47*). More recently, a study using endogenous stable lead isotopes as a tracer of skeletal lead release in environmentally exposed subjects (1–6 μg/dl blood level) indicated that the skeleton contributed from 40–70% of the lead in blood (*16*). This suggests that blood lead levels not only reflect recent exogenous exposures, but that under steady-state conditions they also reflect the release of older accumulated lead from the skeleton.

Several of the above studies are also consistent with a study of autopsy specimens by Wittmers et al. (*58*). In that study, the authors reported that bone lead levels increased linearly with age, but declined after age 40–55 in bone sites that contained a greater amount of trabecular bone, like the vertebra, ilium, and rib. This decline was greatest in women, but a decline was also seen in men. Because hormone depletion-induced increases in bone resorption begin to occur in this age group and preferentially affect trabecular bone (*59*), these data suggest that bone loss in the aged may lead to notable declines in trabecular bone lead content since current environmental exposures are substantially reduced over those just 1–2 decades ago (*2*). The cross-sectional nature of this study limits further interpretation, however, because differences in bone lead could represent an exposure–cohort effect rather than a biological effect. In other words, the older subjects may have had higher bone lead levels than the younger subjects because the lead exposures the older subjects had endured when they were young were higher than the lead exposures currently being endured by the young subjects (exposure differences between age cohorts). This is in contrast to assuming that older and younger subjects had similar lead exposure experiences and hypothesizing that the higher bone lead levels observed among older subjects were due to longer periods of biological accumulation (biological effect). Complementary observations were reported by Silbergeld et al. (*45*) and Symanski and Hertz-Picciotto (*46*) who found that postmenopausal women had significantly higher blood lead levels than premenopausal women, even after controlling for age, race, income, alcohol consumption, and other variables. Based collectively on the above studies, there is compelling evidence that the skeleton may be a significant endogenous source of lead to the circulation across a wide spectrum of age and lead exposure cohorts. However, other than a few case reports of bone lead mobilization during pathologic states involving greatly increased bone turnover such as hyperthyroidism (*47*), tumorous infiltration of bone (*60*), and chemotherapy (*61–63*), few human studies to date have provided evidence that demonstrates an association between bone lead mobilization and toxicity, while no comparable evidence has been published with regard to animal studies.

Inferences on paradigms from KXRF studies. In humans, additional evidence that may shed light on the relative importance of bone lead as repository versus bone lead as source is being generated from research using KXRF. This evidence stems from the ability of KXRF to take separate measurements of lead in cortical and trabecular bone and provide information on the distinct differences between these two types of bones in terms of lead kinetics.

Several studies, including those using radio-tracer (*64*) and standard metabolic methods (*16,26,27,64*), have substantiated the greater importance of trabecular bone over cortical bone as the predominant skeletal source of lead to the circulation under steady-state conditions. This importance is based largely on the elevated blood perfusion and turnover rates of trabecular bone. For example, in a typical individual with a skeletal mass of 6 kg, the ratio of trabecular to cortical bone mass is approximately 1:4. If the lead concentrations of trabecular and cortical bone were 10 mg/kg and 5 mg/kg, respectively [consistent in direction with Wittmers et al. (*58*) and Goldman et al. (*47*) among others], and the release rate of trabecular versus cortical bone were 15%/year versus 3%/year [as suggested by Leggett (*27*)], then the rate of lead release from trabecular bone can be calculated to be 1.8 mg/year, which is almost threefold higher than the value of 0.68 mg/year calculated for cortical bone.

The relative importance of trabecular bone to circulating lead is also based on

investigations using KXRF. In studies of retired lead workers, it was shown that blood lead levels following cessation of exposure correlate with trabecular bone lead levels and not with cortical lead levels (*12,57*). Chelatable lead was found to correlate with lead in biopsies of the vertebrae (a mostly trabecular bone), but not KXRF-measured lead in finger bone (which is mostly cortical bone) (*65*). Fleming et al. (*66*) on the other hand, found that the relationship of KXRF-measured bone lead to blood among lead smelter workers who had not worked for 10 months, as well as retired lead smelter workers, was stronger for the tibia (cortical bone) than the calcaneus (trabecular bone). One potential explanation is that although bone mineral density of the calcaneus has been reported to be a good indicator of overall trabecular bone density (*67*), the error for calcaneal measurements is generally larger than that of tibia measurements, which may have resulted in a more attentuated bone lead–blood lead relationship.

Several recent epidemiologic investigations using KXRF have targeted measurements at the mid-tibia and patella bones, taking advantage of the fact that these two bone sites are almost purely cortical and trabecular, respectively, in nature (the patella measurement may also be influenced by lead concentrations in the distal and proximal ends of the femur and tibia, respectively; both of these bone segments are also predominantly trabecular in nature) (*22*). In a cross-sectional investigation of middle-aged to elderly men, it was found that patella lead was superior to tibia lead in its correlation with blood lead level, even after adjusting for age, race, education, smoking, and alcohol consumption (*68*). Among these mostly retired men, patella lead accounted for 70% of the total variance of blood lead that the final model could explain. In a cross-sectional study of postpartum women in Mexico City, patella lead was superior to tibia lead in its correlation with blood lead levels, even after adjusting for current use of lead-glazed ceramics (one of the main sources of environmental lead exposure in Mexico), consumption of foods high in calcium, years living in Mexico City, education, age, smoking, and parity (*69*).

Thus, existing data suggest that trabecular bone is the predominant bone type providing lead back to the circulation under steady-state and pathologic conditions and that cortical bone lead levels are a better dosimeter of long-term cumulative lead exposure because of the long biologic mean life of lead in that tissue (>10 years) compared to trabecular bone (1–5 yrs) (*26,27*). Significant mobilization of lead from trabecular bone would result in an improved correlation between blood and trabecular bone lead levels in the absence of elevated current exposures, which would be evident in epidemiologic studies investigating these parameters. Of equal importance is investigation of the bone lead as repository paradigm, whereby cortical bone lead levels are determined to evaluate cumulative exposures and their relationship with the presence of past or current (i.e., permanent) toxicity.

Although there are only a handful of studies of bone lead as a predictor of toxicity that employed separate measurements of cortical and trabecular bone, the few that exist provide a range of intriguing and at times apparently conflicting results. For example, in a study of construction workers who had moderate occupational exposures to lead, patella (trabecular) bone lead but not tibia (cortical) bone lead, was associated with declines in hematocrit and hemoglobin (*36*). Similarly, in a case–control study of middle-aged to elderly women, patella bone lead, but not tibia bone lead, was associated with an increased risk of hypertension (*70*). In contrast, however, an investigation of middle-aged to elderly men found that bone lead in the tibia and not the patella was associated with an increased risk of hypertension (*37*). A similar relationship was observed in a study of birth outcomes among women in Mexico City, where lead in the tibia and not the patella was associated with lower birth weight (*38*).

These results suggest that the mechanisms underlying the bone lead as repository and bone lead as source paradigms vary in their relative importance, depending on the measure of toxicity, magnitude and duration of lead exposure, and characteristics (e.g., age, sex) of the subjects. It is also important to recognize, however, that these contrasting results may be due to artifacts of experimental technique. For example, the KXRF measurement uncertainty associated with patella (trabecular) bone measurements is usually greater than that of tibia (cortical) bone, due to a lower relative mineral density. Thus, it should be considered that tibia bone lead may be more predictive of toxicity because of the greater precision of those measurements, rather than the biological importance of cortical bone.

Potential benefits of plasma lead measurements. The degree to which whole blood lead reflects the labile, toxic fraction of lead in the circulation is not well known, although it has been suggested that lead in plasma provides a more kinetically responsive and toxicologically relevant marker of lead than does whole blood lead (*28,71,72*). The limitations of whole blood lead measurements as a marker of lead exposure and, more importantly, as a biomarker of readily labile and toxic lead have been considered for several years (*73*). As indicated above, it is known that the skeleton can contribute lead back to the circulation, although the nature of this relationship across varying exposure regimens and bone metabolic states is not known. We propose that lead in the plasma fraction of the circulation may provide additional (and possibly superior) information on the release of labile, toxic lead from the skeleton.

Until recently, arguments substantiating the need to evaluate plasma lead to investigate bone lead release was based, in part, on previous reports showing little or no correlation between whole blood and plasma lead at low to moderate blood lead levels (e.g., 5–25 µg/dl) (*74–76*). Included in a number of those reports was an apparent severalfold variation in the relative partitioning of lead between whole blood and plasma for a given whole blood lead level. More recently, however, studies [Hernandez-Avila et al., unpublished data; (*77*)] have observed a remarkably well-defined and precise curvilinear relationship between whole blood and plasma lead levels, indicating that individual variation in the relative distribution of lead between whole blood and plasma is not as variable as previously believed. These recent data have provided a basis from which to investigate variation in lead partitioning between whole blood and plasma within individuals and specifically a means to investigate the relationship between bone lead content and plasma–whole blood lead partitioning.

The potential benefit of such an investigation is substantiated by evidence indicating that the blood plasma (vs. whole blood) contains the most labile, biologically active fraction of lead in the circulation (*11,28,71*). Within the circulatory compartment, lead is partitioned primarily between the red cell and plasma (*28,76,79,80*). Nearly all of the lead that enters the body passes through the blood plasma, although based on recent data (Hernandez-Avila et al. unpublished data) from individuals with whole blood lead levels between 2 and 40 µg/dl, only 0.4% ± 0.1 standard deviation (SD) of the whole blood lead is contained within the plasma. Further, studies evaluating plasma lead levels during EDTA chelation have shown that plasma lead reflects the movement and exchange of endogenous lead better than whole blood lead levels (*81–83*). Finally, the data of Cavalleri and Minoia (*84*) on plasma and whole blood lead levels following occupational exposures suggest that the partitioning of lead in plasma may vary depending on the type/source (i.e., chemical speciation) of exposure. Thus, plasma lead levels may better reflect exposures to lead of different

chemical species, which may be of relevance here if lead mobilized from the skeleton into the circulation exists as a different bimolecular species compared to lead derived from recent exogenous exposures.

A recently completed study of serum lead that also employed KXRF to measure bone lead levels bears directly on this issue. Cake et al. (*71*) measured serum lead, whole blood lead, and lead in both the tibia (chiefly cortical bone) and calcaneus (chiefly trabecular bone) in each of 49 active lead workers. Serum lead was measured by an inductively coupled plasma mass spectrometer in conjunction with an isotope dilution standardization method developed by Bowins and McNutt (*85*). Serum lead measurements were found to correlate more strongly with both *in vivo* bone lead measurements (tibia and calcaneus) than did whole blood lead. The ratio of serum lead to whole blood lead varied from 0.8 to 2.5% and showed the strongest correlation with lead in the calcaneus (trabecular bone).

This study further substantiates the proposed benefit of plasma (or serum) lead measurements for evaluating exchange and release of lead from the skeleton. It also is consistent with other studies, which suggest that plasma lead levels reflect the biologically labile component of circulatory lead better than does whole blood. The quantitative importance of this kinetic paradigm can be illustrated if one takes the example of a typical blood sample in which the plasma lead concentration is 0.1 µg/dl, the red blood cell concentration is 10 µg/dl, the hematocrit is 40%, and the consequent whole blood lead concentration is 4.06 µg/dl (Fig. 2). A threefold rise in plasma lead concentration to 0.3 µg/dl would greatly increase the lead available to cross cell membranes; however, the increase in whole blood lead concentration (to 4.18 µg/dl) concentration would be negligible.

Despite the apparent benefit of measuring plasma (or serum) lead levels in the evaluation of endogenous lead movement and exchange, few studies have demonstrated the capability for routinely making those measurements. The primary reasons for this are the difficulties associated with avoiding lead contamination during the collection, processing, and analyses of clinical samples that may contain <0.05 µg Pb/dl and the difficulties associated with performing analyses with the accuracy and precision necessary to suitably evaluate interindividual or interpopulation differences in lead levels (*71,72,75,86*). In addition to the paramount concern for sample lead contamination from external sources, there is also concern for lead contamination of plasma (or serum) from hemolysis during blood collection and separation, as recently demonstrated (*78*). Because lead in blood is partitioned predominantly (>98%) with erythrocytes, the hemolysis that often occurs during blood collection and separation may contribute relatively substantial amounts of lead to the separated plasma (or serum) fraction, even in cases where noticeable hemolysis (i.e., reddening of plasma) does not occur. This concern has not yet been systematically evaluated although, together with external lead contamination, it may be an important contributor to the amount and variability of lead in plasma reported in many studies. An additional technical concern involves standardizing the protocol in terms of time of day and fasting because the plasma is a small and rapidly exchangeable lead compartment. Even small discrete exposures, such as a meal, may appear as temporal spikes in the plasma and urine; thus, standardizing the timing of sample collection is of importance to measuring basal plasma lead levels.

Strategies for distinguishing between paradigms. To identify the relative importance of the two paradigms by which bone lead may serve as a biological marker of exposure and toxicity, several strategies may be useful. First, animal studies may be undertaken that can shed insight both into the biokinetics of lead concentrations in bone, plasma, blood, and target organs, as well as the relative impact of lead dose biomarkers in predicting forms of toxicity. Radioisotope tracer methods comparing stable isotope ratios of lead in plasma, bone, and blood may also prove useful. Toxicity studies would only be useful in so far as animal models have been developed for such outcomes, which could be said, for example, of hypertension (*87*) and neurobehavioral effects (*88,89*). Animal studies should use environmentally relevant lead exposure regimens. Excessively elevated acute or chronic exposures may themselves result in direct permanent damage to lead-sensitive target organs, thereby compromising the ability to evaluate secondary toxicity due to mobilized bone lead. Suitable animal models also exist for various pathologies of skeletal physiology (e.g., hormone depletion-induced osteopenia) that could shed light on whether bone turnover modifies the toxicity of lead released from bone.

Epidemiologic studies are, of course, time consuming and costly and mostly limited to observational investigations. However, using KXRF to measure bone lead and incorporating biological markers of bone resorption and/or turnover, as well as densitometry methods, could significantly enhance the ability of epidemiologic studies to provide insights in this area. Data indicating that high bone turnover increases the amount of toxic lead released from bone would constitute prima facie evidence that bone lead as source is an important mechanism of toxicity. Ideally, clinical studies should be prospective in design and include subjects that represent a broad range of lead exposures to evaluate rates of lead accumulation in bone. In cases where this is impractical, cross-sectional designs may be employed, taking into account, if necessary, different baseline exposure levels suffered by the older subjects when they were younger.

Identifying a Marker for Bone Lead Mobilization

In considering these types of studies, another issue that arises is identifying metrics that can be used to estimate bone lead mobilization. On first glance, it would seem that by taking repeated *in vivo* measures of bone lead levels in a longitudinal design, we may assume that declines in bone lead directly reflect mobilization of bone lead into the circulation. However, because KXRF instruments measure bone lead levels relative to bone mineral, concurrent changes in bone mineral content must also be considered. For instance, a bone site that is primarily undergoing mineral resorption and little formation would experience similar declines in bone mineral and bone lead, with little or no apparent net change in bone lead concentration (in micrograms Pb per gram bone mineral) (Fig. 3B). Thus, the absence of a decline

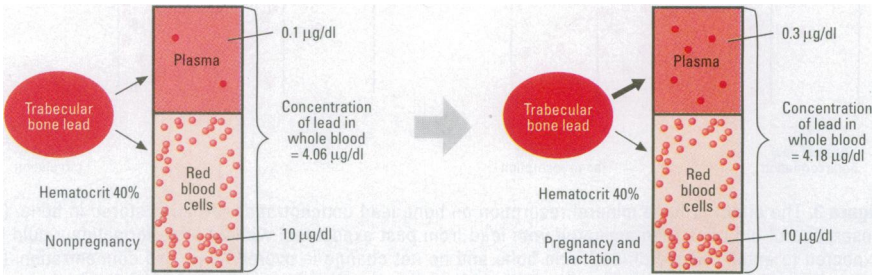

Figure 2. Paradigm of lead kinetics showing how a threefold rise in plasma lead concentration without a rise in red blood cell lead concentration would cause a negligible rise in whole blood lead concentration.

in bone lead concentration at the measured site would be incorrectly interpreted as no net loss of bone lead. (This scenario assumes that both bone mineral and bone lead are resorbed and released from bone with equal efficiency, although this is not actually known.) Conversely, a net decline in bone lead concentration would occur during mineral turnover if there were a simultaneous formation of bone mineral that was relatively deplete of lead (because of low external lead exposure and therefore low circulating lead) and resorption of bone mineral that was relatively rich in lead (Fig. 3C).

Within this conceptual framework, measurement of bone mineral density could provide some benefit because mineral density reflects the net balance of both bone formation and resorption. The dynamic relationship between bone formation and resorption must be considered when interpreting measured changes in bone density since apparent reductions in mineral density can arise from both a reduction in formation with ongoing resorption, ongoing formation with increased resorption, or a combination of the two—all of which could result in different apparent versus actual amounts of bone lead release.

Assuming that the mobilization of bone lead into the circulation is the parameter of most interest to be measured, a specific marker of bone resorption should be measured. Several urinary markers of bone resorption are available, including urinary calcium, hydroxyproline (HYP), pyridinoline cross-links (PYD), and, most recently, the cross-linked *N*-telopeptide of type I collagen (NTX). Among these, urinary NTX appears to be the most promising because of evidence indicating its specificity for bone turnover. Although calcium, HYP, and PYD are components of bone, all of these markers are found in tissues other than bone (*90–92*). HYP also undergoes metabolism in the liver (*91,92*), rendering levels in urine even less reflective of bone resorption. Urinary NTX, on the other hand, is derived specifically from bone collagen degradation and is not metabolized (*93*). Rosen et al. (*94*) recently demonstrated that creatinine-adjusted urinary NTX levels are more responsive to acute thyroid hormone-induced increases and bisphosphonate-induced decreases in bone resorption than either PYD or HYP, thereby supporting NTX levels as the most accurate and specific biological marker of bone turnover available.

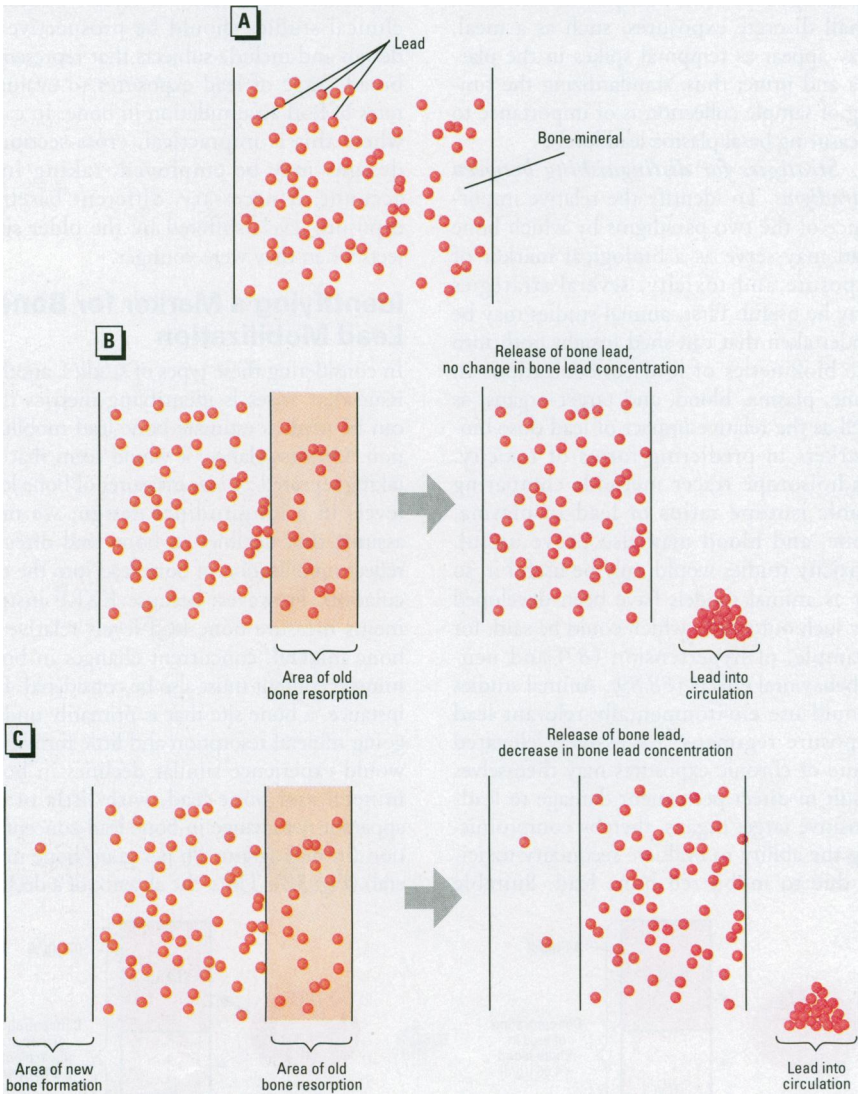

Figure 3. The effect of bone mineral resorption on bone lead concentration. (A) Lead stored in bone. (B) Resorption of old bone (contaminated with lead from past exposures) without bone formation would be expected to entail release of lead from bone and no net change in overall bone lead concentration. (C) Resorption of old bone (contaminated with lead from past exposures) with formation of new bone that is relatively deplete in lead (because of low current lead exposures) would be expected to entail release of lead from bone and a net decline in overall bone lead concentation.

Summary and Conclusions

Several lines of evidence have converged to indicate that skeletal lead burden may be a biological marker of lead dose that is more useful than whole blood lead in predicting some forms of toxicity arising from chronic low to moderate lead exposure. KXRF has emerged as a valuable tool for making *in vivo* measurements of skeletal lead in epidemiologic studies. Here, we have advanced two conceptual paradigms, the bone lead as repository for cumulative lead exposure paradigm and the bone lead as source paradigm, to explain how skeletal lead burden may serve as a predictor of chronic toxicity. If the latter paradigm predominates over the former, factors that influence bone turnover, and therefore bone lead mobilization, may modify the toxicity of lead that has accumulated in the skeleton over time. Strategies for distinguishing the relative importance of the two paradigms in epidemiologic studies include making separate measurements of trabecular and cortical bone lead and incorporating markers of bone turnover and measurements of bone mineral status. In addition, applying techniques for measuring lead in plasma may be important in so far as plasma lead may represent a more toxicologically relevant component of circulating lead than does whole blood, with a special relationship to bone lead stores that may help to explain the mechanism(s) of bone lead toxicity. Further research in these areas is under way.

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